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Artificial Zinc Finger Peptide Containing a Novel His_4 Domain

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Zinc finger constitutes one of the most common DNA binding motifs. Although zinc finger proteins consisting of Cys_2His_2, Cys_3His, Cys_4, and Cys_6 domains are known in nature, a novel His_4 zinc finger protein has never been observed. Herein, we have created the first artificial His_4-type zinc finger protein (H_4Sp1) engineered by Cys to His mutations of the Cys_2His_2-type zinc finger transcription factor Sp1. The CD features of the single finger H_4Sp1f2 and three-finger H_4Sp1 clearly demonstrate the folding of the mutant His_4 peptides by complexation with Zn(II). The NMR study of Zn(II)-H_4Sp1f2 reveals that some distortions of the helical region occur due to Zn(II) coordination. The gel mobility shift assay and DNase I footprinting analysis strongly show the binding of Zn(II)-H_4Sp1 to the GC-box site of duplex DNA. The methylation interference pattern of Zn(II)-H_4Sp1 binding significantly resembles that of the corresponding C_2H_2Sp1 binding. The present artificial peptide H_4Sp1 is the first example of a zinc finger containing the His_4 domain. Of special interest is the fact that the zinc finger domains of H_4Sp1 are folded (although not identical to the native structure) and bind DNA similar to wild type C_2H_2Sp1.

Key words: Zinc finger/ Sp1/ Designed metallofinger/ DNA binding

Metalloproteins play important roles in gene regulation, and some metal ions also participate in the transcriptional stage by protein-mediated metallation. In particular, zinc ion is essential as a structural factor for zinc finger proteins, which constitute one of the most common DNA binding motifs. Zinc finger proteins acquire DNA binding ability by Zn(II)-complexation. In nature, Cys_2His_2-, Cys_3His-, Cys_4-, and Cys_6-type zinc fingers exist. Among them, the Cys_2His_2-type zinc finger motif especially possesses the following fascinating characteristics; (1) a compact ββα fold is acquired by Zn(II)-coordination to bind the asymmetric DNA sequence, (2) one finger recognizes 3 to 4 base pairs by the side chains of amino acids located on the recognition helix, and (3) extended recognition can be attained by tandem repeating. The mini ββα fold of the Cys_2His_2-type zinc finger motif is an attractive framework for designing a novel metallofinger motif. At the present stage, no newly designed metallofingers that have evident DNA recognition ability are known. In addition, zinc finger proteins containing the His_4 domain have never been found or engineered. Herein, we have created the first novel His_4-type zinc finger protein by Cys to His mutations of a Cys_2His_2-type zinc finger in transcription factor Sp1.

In order to clarify the structural alteration of the zinc finger domain by Cys to His mutations, the H_4Sp1f2-Zn(II)

BIOORGANIC CHEMISTRY — Bioactive Chemistry —

Scope of research

The major goal of our laboratory is to elucidate the molecular basis of the activity of various bioactive substances by biochemical, physicochemical, and synthetic approaches. These include studies on the mechanism of sequence-specific DNA cleavage by antitumor or carcinogenic molecules, studies on the DNA recognition of zinc-finger proteins, and model studies on the action of ion channels. In addition, artificial designed peptides have also been developed as useful tools in molecular biology and potentially in human medicine.

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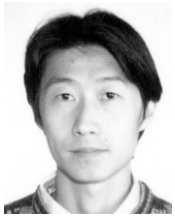

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complex was investigated using two-dimensional NMR techniques. In contrast to C_2H_2Sp1f2, a 3D structural model of H_4Sp1f2 showed the twisting of an extended strand in the Pro2-Trp7 region and helical unwinding around Asp18-Gln21, as indicated by the disappearance of the $C_\alpha H_i$-NH_{i+3} NOEs between them. The twisting detected in the Pro2-Trp7 region probably occurs in order to accommodate a tetrahedral binding geometry enforced by zinc coordination constraints.

To examine whether the folding of H_4Sp1 is Zn(II)-dependent as well as that of H_4Sp1f2, CD studies were carried out. The mutant protein H_4Sp1 was constructed from C_2H_2Sp1 by Cys to His mutations of all three of the zinc finger domains. The CD spectrum of H_4Sp1 in the Zn(II)-free buffer was characteristic of the random coil. In the presence of Zn(II), the negative CD signal bands near 203 nm and 222 nm clearly increased, demonstrating the folding of H_4Sp1 by Zn(II)-complexation. The CD feature of Zn(II)-H_4Sp1 was different from that (207 and 226nm) of Zn(II)-C_2H_2Sp1 to some degree, suggesting that the zinc finger domain of H_4Sp1 did not form the structure identical to that of the wild type C_2H_2Sp1.

The results of the gel mobility shift assays clearly showed that Zn(II)-H_4Sp1 was bound to the DNA fragment (41 bp) containing an Sp1 recognition site GC box (5'-GGGGCGGGGCC-3'). From the evidence for the monomeric binding of the C_2H_2-type zinc finger to the single binding site, the binding mode of Zn(II)-H_4Sp1 is also monomeric because the mobility of the shifted band was the same as that of Zn(II)-C_2H_2Sp1. However, the DNA binding affinity of H_4Sp1 for the GC box was lower than that of C_2H_2Sp1. Furthermore, the Zn(II)-dependent DNA binding ability of H_4Sp1 was shown by the decrease in the DNA binding by EDTA. From the result of the DNase I footprinting analysis of Zn(II)-H_4Sp1 for a 148-bp DNA fragment containing the GC box, H_4Sp1 almost protected the residues of the GC box at 2.4 μM from DNase I cleavage in the presence of Zn(II). These results strongly indicate that the H_4Sp1 still retained the DNA binding ability in spite of the Cys to His mutation.

Previous studies have demonstrated that C_2H_2Sp1 can bind DNA by interacting with Co(II), Cd(II), and Ni(II) as well as Zn(II). To clarify whether the present H_4Sp1 gains the DNA binding ability by various metal complexations, H_4Sp1 was reconstructed with Zn(II), Ni(II), Cu(II), Co(II), or Cd(II) from insoluble fractions during the purification step, and a gel mobility shift assay was conducted. While Zn(II)-reconstituted H_4Sp1 was really bound to a GC-box fragment, under this experimental condition, the DNA binding activity was not detected in the case of Ni(II)-, Cu(II)-, Co(II)-, and Cd(II)-reconstituted H_4Sp1. From the CD studies of the H_4Sp1f2, only the Zn(II) binding to its His_4 domain highly induced an ordered conformation with the secondary structure. Therefore, these results indicate that effective folding by Zn(II) binding is essential for the DNA binding ability of H_4Sp1.

When Zn(II)-H_4Sp1 was bound to the GC-box fragment (41 bp), its methylation interference pattern was compared with that of Zn(II)-C_2H_2Sp1. In the case of H_4Sp1, strong base contacts with G(2), G(3), G(4), and G(6) in the guanine-rich strand (G-strand) and G(5') in the cytosine-rich strand (C-strand) were detected and also weak contacts with G(1) and G(7) in the G-strand and G(11') in the C-strand were observed. The interference feature remarkably resembled that of the wild type C_2H_2Sp1, indicating similar DNA binding modes between H_4Sp1 and C_2H_2Sp1.

The Cys to His conversion generates a larger metal coordination sphere because the size of the His residue is larger than that of the Cys residue as opposed to the case. Although the Cys to His mutation results in a decreased DNA binding affinity, the present peptide H_4Sp1 maintains the DNA binding ability. In spite of the structural alteration or distortion of the zinc finger domain as clearly indicated by the CD and NMR evidence of H_4Sp1f2, it is of special interest that specific interactions between the recognition helix of the finger 2 and its subsite, 5'-GCG-3', are retained. In addition, fingers 1 and 3 of H_4Sp1 also preserve the DNA recognition ability of the wild type C_2H_2Sp1. Several reasons are considered for the fact that H_4Sp1 and C_2H_2Sp1 show similar DNA recognition, despite the somewhat different helical conformation between their zinc finger domains. First, the DNA binding to Zn(II)-H_4Sp1 may cause an additional structural change and result in a specific DNA recognition analogous to Zn(II)-C_2H_2Sp1. Indeed, it is known that the specific DNA binding induces a structural alteration in the basic region of GCN4. Second, Zn(II)-H_4Sp1 may recognize DNA bases in a different manner from Zn(II)-C_2H_2Sp1. Namely, the mutant peptide H_4Sp1 compensates for specific contacts with particular bases by a directional change in the side chain that is essential for DNA recognition. As a result, the recognition helix of Zn(II)-H_4Sp1 may be located in a different orientation to DNA from that of Zn(II)-C_2H_2Sp1.

In conclusion, this paper describes the preparation, structure, and DNA binding properties of an engineered His_4 mutant of the zinc finger protein Sp1. We present the NMR structure for a single mutated zinc finger (H_4Sp1f2) from Sp1 and the DNA binding data for a three domain mutant (H_4Sp1). The CD characteristics of H_4Sp1f2 and H_4Sp1 are also compared with those of C_2H_2Sp1f2 and C_2H_2Sp1. Interestingly, the His_4 domain is folded and recognizes the GC-box DNA. The development of such a novel peptide may also lead to a new zinc finger type or other metal finger domain. Additionally, the present results suggest that a His_4-type zinc finger protein may also exist in nature.